Reference: *Biol. Bull.* **205:** 121–132. (October 2003)

Larval Development and Metamorphosis in *Pleurobranchaea maculata*, With a Review of Development in the Notaspidea (Opisthobranchia)

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Abstract. *Pleurobranchaea maculata* is a carnivorous notaspidean that is common in New Zealand. This species produces small eggs (diameter 100 μm) and planktotrophic veligers that hatch in 8 d and are planktonic for 3 weeks before settling on biofilmed surfaces (14 °C). Larval development is known in detail for only two other notaspidean species, *P. japonica* and *Berthellina citrina*. In all three species of pleurobranchids, mantle and shell growth show striking differences from veligers of other opisthobranch taxa. In young veligers of pleurobranchids, the shell is overgrown by the mantle, new shell is added by cells other than those of the mantle fold, and an operculum does not form. Thus some "adult" traits (*e.g.*, notum differentiation, mechanism of shell growth, lack of operculum) are expressed early in larval development. This suggests that apomorphies characteristic of adult pleurobranchids evolved through heterochrony, with expression in larvae of traits typical of adults of other clades. The protoconch is dissolved post-settlement and not cast off as occurs in other opisthobranch orders, indicating that shell loss is apomorphic. *P. maculata* veligers are atypical of opisthobranchs in having a field of highly folded cells on the lower velar surface, a mouth that is posterior to the metatroch, and a richly glandular, possibly chemodefensive mantle. These data indicate that notaspidean larvae are highly derived in terms of the novel traits and the timing of morphogenic events. Phylogenetic analysis must consider embryological origins before assuming homology, as morphological similarities (*e.g.*, shell loss) may have developed through distinct mechanisms.

Received 27 August 2002; accepted 23 June 2003.
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Introduction

The Notaspidea is a small, specialized order of opisthobranchs that are considered to be phylogenetically intermediate between the highly derived Nudibranchia and the more basal Cephalaspidea (Schmekel, 1985; Willan, 1987; Mikkelsen, 1998) or monophyletic with the Nudibranchia to form the Nudipleura (Wägele and Willan, 2000). Comparative embryological studies of the Notaspidea are therefore significant for phylogenetic analyses but also for understanding morphological evolution in the Opisthobranchia, a clade rich in homoplastic similarities (Gosliner, 1991, 1994; Mikkelsen, 1998). Unfortunately, little is known about notaspidean development. My goal is to describe larval development in *Pleurobranchaea maculata* (Quoy and Gaimard, 1832) and to provide a preliminary analysis of development in Notaspidea.

Adult notaspideans are carnivores and opportunistic scavengers. They are characterized by a single, external ctenidium on the right side, rolled rhinophores, and a flattened shell (Willan, 1983; Schmekel, 1985; Willan, 1987). The order traditionally includes the Umbraculomorpha (families Tylodinidae and Umbraculidae) with large limpet-like external shells and a small mantle, and the Pleurobranchomorpha (Pleurobranchidae) with a prominent mantle and shells that are internal and reduced or lost in adults (Thompson, 1976; Willan, 1983, 1987). Mantle secretions provide chemical defense in many species, both shelled and shell-less, through the release of acid (Thompson and Slinn, 1959; Thompson, 1988), secondary metabolites (Ciavatta *et al.*, 1993, 1995; Spinella *et al.*, 1997), or dietary alkaloids (Ebel *et al.*, 1999). On the basis of adult anatomy, some investigators suggest that the Notaspidea are polyphyletic (Schmekel, 1985), while others argue that they are

paraphyletic and require inclusion of the Nudibranchia (Wägele and Willan, 2000).

Phylogenetic analysis promotes an understanding of the relatedness among taxa, and of morphological evolution, for which comparative developmental data are essential. Although there is no reason to assume that larval and adult traits have coevolved, inclusion of larval traits strengthens analyses by increasing the data set, but more importantly, by revealing homologies in traits with similar embryological origins. While we have an abundance of data on nudibranchs, development is poorly known for some of the less speciose orders. For the Notaspidea in particular, detailed descriptions of larval development are limited to two species of Pleurobranchidae: *Berthellina citrina* Rueppell and Leuckart, 1828 (Gohar and Abul-Ela, 1957; Usuki, 1969), and *Pleurobranchaea japonica* Thiele, 1925 (Tsubokawa and Okutani, 1991). Partial information on larvae is available for less than a half-dozen additional species. Even these limited data suggest that larval pleurobranchids are diverse and can be planktotrophic, lecithotrophic, or direct developing (Gohar and Abul-Ela, 1957; Usuki, 1969; Thompson, 1976; Tsubokawa and Okutani, 1991; Wägele, 1996; Goddard, 2001b). We lack descriptions of development for all species of the Tylodinidae and Umbraculidae.

My objectives are to describe development of *Pleurobranchaea maculata*, and to compare development of notaspideans with other opisthobranchs. *P. maculata* is an opportunistic carnivore found in New Zealand, southeastern Australia, China, Sri Lanka, and Japan (Willan, 1983; Marcus and Gosliner, 1984). Notes by Willan (1983) indicate that *P. maculata* produces long, cylindrical egg masses that release planktotrophic veligers. The present study extends this record to include embryology, larval structure, and metamorphosis using bright field and scanning electron microscopy (SEM).

Materials and Methods

Specimens of *Pleurobranchaea maculata* were collected from intertidal sandflats at the Tahuna Torea Reserve, Tamaki Estuary, Auckland, New Zealand, in August 2001. Adults were maintained at ambient conditions (seawater temperature 14 °C) and were fed cockles (*Austrovenus stutchburyi* Wood, 1828) or oysters (*Ostrea lutaria* Hutton, 1873) daily. Egg masses were cultured in flowing seawater (14 °C), and small sections were removed for observation. Larvae were cultured in 1-l jars of 1-μm-filtered seawater at 17–19 °C. Larvae were fed a 1:1:1 mixture of *Dunaliella salina* Teodoresco, 1905, *Isochrysis galbana* Parke, 1949, and *Pavlova lutheri* Green, 1975 three times weekly. Settlement occurred in pediveligers that were cultured in a 1-l jar (17–19 °C) with a 1-week growth of biofilm on the glass surface.

Live embryos, larvae, and juveniles were observed, sketched, and photographed with a Nikon AFX photomicroscope. Measurements were taken to the nearest 5 μm. Pediveligers and early juveniles were fixed for SEM in 2.5% glutaraldehyde followed by post-fixation in 1% osmium tetraoxide, both in filtered seawater. After fixation, specimens were dehydrated in ethanol, critical-point-dried with a Polaron B3000 Series II critical-point drier, and coated with gold-paladium with a Polaron SC7640 sputter coater. Specimens were observed with a Phillips XL 30S SEM at 5 kV. Plates were composed in Corel Photo Paint 9.0 and Corel Draw 9.0, using both scanned negatives (light micrographs) and digital images (electron micrographs).

Results

The cylindrical egg masses were 171.60 ± 59.61 mm in length (X ± SD; $n = 5$; range 88–240 mm) and about 8–12 mm in width, as produced by two adults 110 and 145 mm in length. *P. maculata* spawned frequently, and these two individuals produced 15 egg masses in about 5 weeks of laboratory culture. Usually adults deposited the loosely coiled egg strings on the side walls of the tank near the air-water interface. The eggs were white, 100.91 ± 2.18 μm in diameter ($n = 45$), and deposited in egg capsules housed within long strings that formed a double spiral at the periphery of the jelly mass. Capsules were 259.50 ± 10.92 by 250.00 ± 10.54 μm in size ($n = 20$), and contained an average of 3.97 ± 1.02 eggs per capsule (range 2–6; $n = 30$ capsules in each of 7 egg masses). Small globules of yolk (filled with lipid droplets and several times larger than polar bodies) detached from developing young in about 18% of the egg capsules observed ($n = 244$ capsules, from a total of 8 egg masses). Early veligers ingested this extra-embryonic yolk before hatching. Fecundity was estimated to be 318,417 ± 110,602 embryos per egg mass ($n = 5$). Egg masses were as figured by Willan (1983).

Spawning generally occurred over a period of 2–3 h in the early and mid-morning. A chronology of early embryological events is summarized in Table 1. Elongate trochophores, with a flat velar field and small pedal rudiment, developed by 4 d. The shell was visible by 4.5 d and had grown to cover the visceropallial mass by 5 d. Also at 5 d, the statocysts, each with a single statolith, were visible, and embryos were capable of ingesting extra-embryonic yolk. By 5.5 d, the dark red pigmented mantle organ was visible and the internal organs, although still yolky, were better defined. The elongate foot lacked an operculum, and velar cilia were capable of metachronic beating and reversals. By 6 d, the internal organs, including the pigmented mantle organ, were sharply defined. The larval retractor muscles, visible at 6 d, were functional by 7 d although the embryos were capable of incomplete retraction only, leaving the velar lobes and foot partially exposed.

Table 1

Summary of the major events in embryonic development of Pleurobranchaea maculata *at 14 °C*

Time (d, h)	Event
0 h	Spawning
2.5 h	1st polar body
4.5 h	2nd polar body
8.5 h	1st cleavage
9.15 h	2nd cleavage
9.45 h	3rd cleavage
22 h	Morula
24–48 h	Blastula formation
48–72 h	Gastrulation
4 d	Trochophores: pedal rudiment and velar field present, embryo elongate
4.5 d	Cap-like shell visible
5 d	Early veligers: larval shell complete, pigmented mantle organ, statocysts, right and left digestive gland present, foot visible but lacks an operculum
6 d	Pigmented mantle organ well developed, retractor muscle present but not functional, digestive glands becoming more distinct
7 d	Retractor muscle functional, velar lobes large, digestive glands still yolky but yolk depleted from stomach, mantle fold well developed
8 d	Hatching: subvelar ridge prominent, kidney rudiment present, pigmented mantle organ, mantle edge rounded, shell surface granulated. Eyes and operculum lacking

Spawning occurred over a 2–3-h period. Timing of early events is relative to the onset of oviposition.

Veligers hatched at 8 d, with a shell length of 135 ± 5.5 μm (n = 20), and began feeding on phytoplankton immediately (Table 1, Fig. 1A). The digestive glands were still yolky and the ciliated stomach had hyaline rods in the posterior wall, as described in nudibranchs (Thompson, 1959). Just above the pigmented mantle organ was a small, transparent organ, presumably the rudiment of the definitive kidney (Gohar and Abul-Ela, 1957). The kidney rudiment remained in close association with the pigmented mantle organ and anus throughout larval development. Larvae lacked eyes at hatching, and the finely ciliated foot lacked an operculum throughout development. The Type 1 shell (types by Thompson, 1961) lacked pigment and was finely granulated on the surface; ridges, as occur in *P. japonica* (Tsubokawa and Okutani, 1991), were not observed.

One week after hatching, the larval shell was about 190 ± 15 μm (n = 20) in length, and the velar lobes had increased substantially in size (Fig. 1B, C, D). New sensory structures had formed, including a pair of black eyes and the pedal tuft, a prominent cluster of elongate cilia on the tip of the foot. The stomach was greatly enlarged relative to shell size, and the hyaline rods were more numerous in the posterior stomach wall. The larval heart was also present and beating. The pigmented mantle organ, now black, was larger (Fig. 1B, D), and the kidney rudiment was much larger, slightly bilobed, and lacking any visible contents. Buds of the rhinophores were present on the anterior velar field (Fig. 1D).

Also in the first week of larval life, the mantle began to envelope the larval shell by growing up and over the dorsal shell aperture (Fig. 1B). The larval shell continued to grow, concurrent with overgrowth by the mantle, during larval development. The veliger was unable to completely retract into the shell, possibly because of the size of the velar lobes and also because the growth of the mantle reduced the effective shell aperture.

About 3 weeks after hatching, the first pediveligers settled on the biofilmed surface of the culture container. The shell, 480 ± 23 μm in length (n = 20), was almost completely covered by the thick, glandular mantle (Fig. 2A–F). The growth zone of the mantle was thin as it extended over the shell, while older mantle tissue became thickened as the mantle glands developed (Fig. 2C). The mantle glands invaginated from the epidermis to the shell margin, forming elongate, simple tubular glands (Fig. 2D, E). Small tufts of cilia were scattered over the mantle surface, between the openings of the mantle glands (Fig. 2E). A lateral ciliary tract was present externally from the opening of the prebranchial aperture on the right side of the "neck" and along the upper right side of the foot (Fig. 2F). The densely ciliated mouth was located ventral and posterior to the subvelar ridge (Fig. 2F). The foot was well developed and covered with fine cilia ventrally and with tufts of cilia on the lateral and upper surfaces (Fig. 2F). The pedal tuft, as described for veligers 1 week after hatching, was absent.

In late-stage larvae and pediveligers, the velum showed additional structures on both the upper and lower surfaces. The rhinophores extended from the upper velar surface as two curved ridges of tissue that were covered with fine cilia (Fig. 2A, F). As the rhinophores developed, they grew anteriorly, then laterally; but they remained open on the lateral surface, thus giving rise to the scroll-like morphology that is typical of adults (Willan, 1983). The oral veil was also visible as a broad, ciliated ridge on the upper surface of the velum, located immediately ventral to the rhinophores but not connected to them (Fig. 2F). The lower velar surface was covered by large (~15 μm in diameter) rounded cells with a highly folded, microvillar surface (Fig. 2F, G). These post-velar cells were tightly packed together and covered the lower surface of each velar lobe from the subvelar ridge to the body wall.

Some internal organs were difficult to observe in live pediveligers because of the thick mantle. The eyes and statocysts were well developed, and the buccal mass was prominent in the anterior digestive tract (Fig. 2A). The pigmented mantle organ was darker, and the enlarged, transparent kidney was easily observed though the thick mantle tissue (Fig. 2B, D).

Acquisition of a juvenile morphology occurred gradually

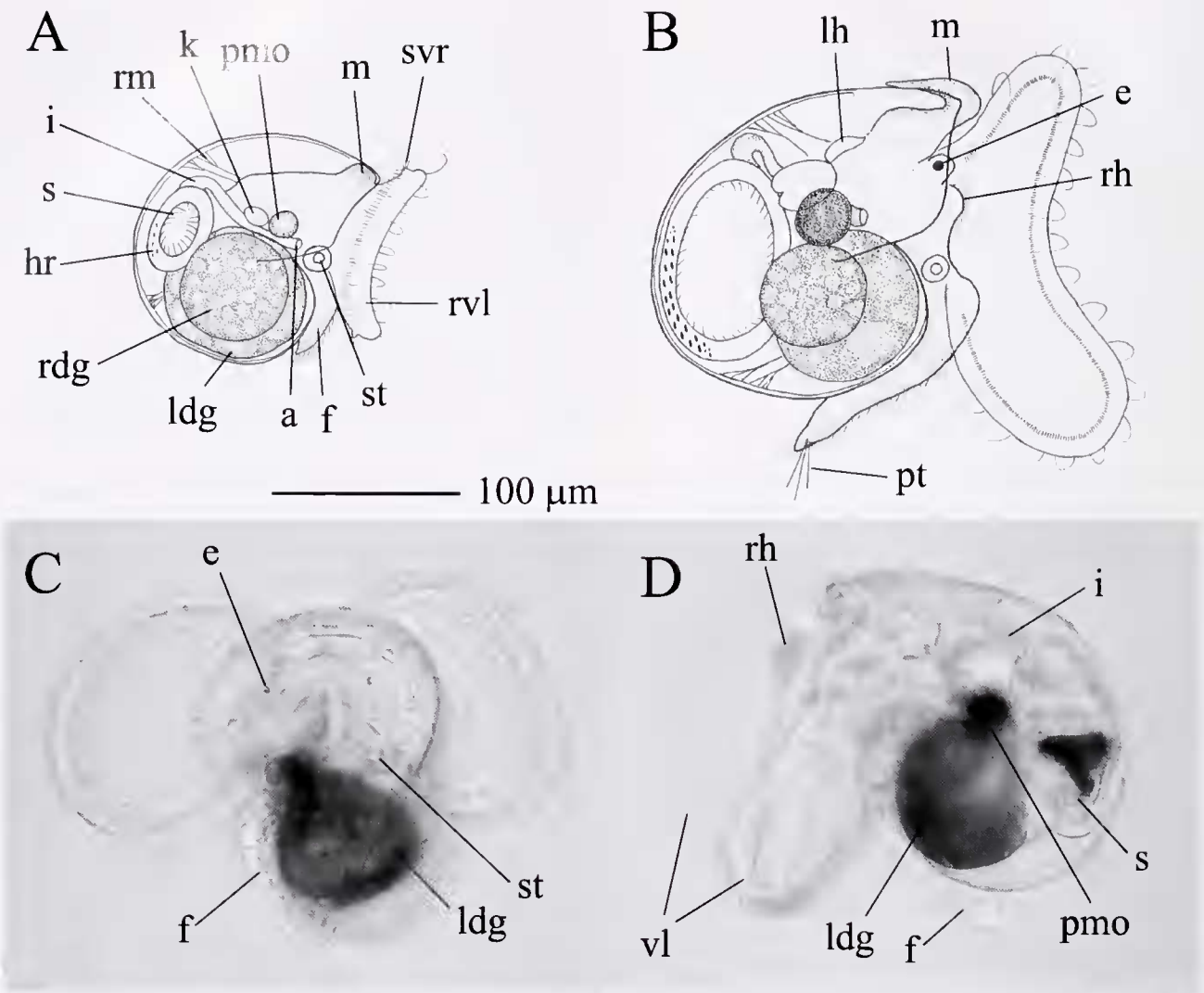
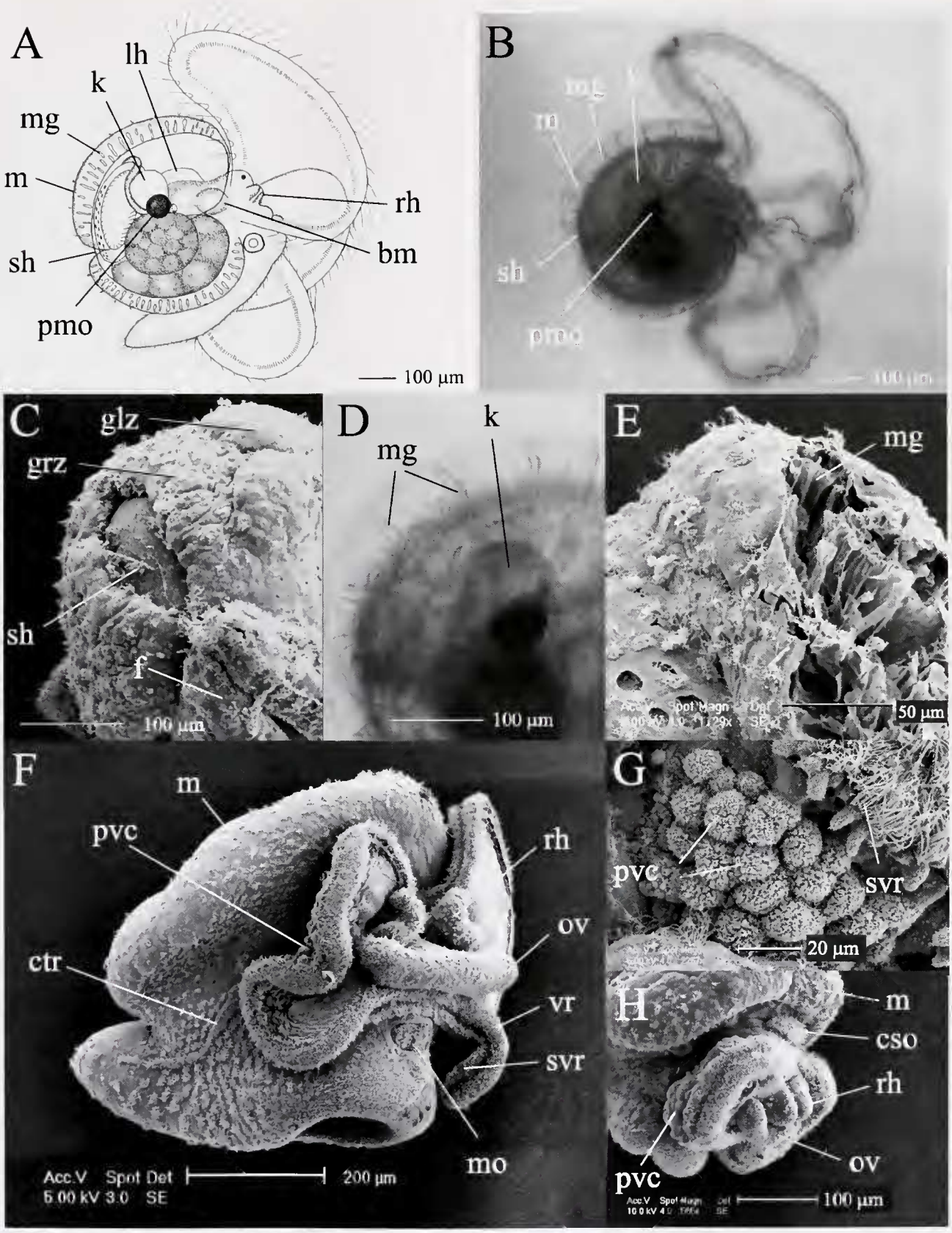


Figure 1. Early veligers of *Pleurobranchaea maculata*. (A) Veliger on the first day of hatching, drawn from life. (B) Veliger 7 d after hatching, drawn from life. (C, D) Bright field micrographs of veligers 7 d after hatching. Scale bar is the same for all four illustrations. a, anus; e, eye; f, foot; hr, hyaline rods; i, intestine; k, kidney rudiment; ldg, left digestive gland; lh, larval heart; m, mantle; pmo, pigmented mantle organ; pt, pedal tuft; rdg, right digestive gland; rh, rhinophore bud; rm, retractor muscle; rvl, right velar lobe; s, stomach; st, statocyst; svr, subvelar ridge; vl, velar lobes.

and involved development of the mantle throughout most of larval life. The final stage of metamorphosis primarily involved loss of the velum. As the velar and subvelar cilia were shed, the highly folded surface of post-velar cells became smooth and the cells were gradually resorbed into the lower velar surface, followed by resorption of the velar

Figure 2. Pediveligers of *Pleurobranchaea maculata*, 3 weeks after hatching. (A, B) Drawing and bright field micrograph of live pediveligers. (C) Scanning electron micrograph of the overgrowth of the larval shell by the mantle. (D, E) Mantle glands in bright field and scanning electron micrographs. (F) Scanning electron micrographs of a pediveliger. (G) Scanning electron micrograph of the post-velar cells on the lower velar surface; the subvelar ridge is shown in the upper right. (H) Scanning electron micrograph of the partially resorbed velar lobes during metamorphosis. bm, buccal mass; cso, cephalic sensory organ; ctr, lateral ciliary tract; f, foot; glz, glandular zone; grz, growth zone; k, kidney rudiment; lh, larval heart; m, mantle; mg, mantle gland; mo, mouth; ov, oral veil; pmo, pigmented mantle organ; pvc, post-velar cells; rh, rhinophore; sh, shell; svr, subvelar ridge; vr, velar ridge.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lobes into the head (Fig. 2H). Early juveniles retained lateral remnants of the velar lobes for several days (Fig. 3A–D). Loss of the velum revealed the cephalic sensory organ, a prominent ciliated organ located dorsally between the two velar lobes (Fig. 2H). The rhinophores extended anteriorly from the remnant of the velar field (Fig. 3A, C), and the oral veil projected as a broad ridge to cover the mouth and anterior foot (Fig. 3A–C). The prebranchial aperture, open on the right side, led to the lateral ciliary tract. The gill had not yet formed (Fig. 3D). The opaque mantle, both glandular and also with a scattering of red pigment, made it difficult to determine when the shell was dissolved, although the buccal mass and digestive glands were easily observed through the ventral body wall (Fig. 3B). The pigmented mantle organ appeared to be lost during late metamorphosis. The kidney remained next to the prebranchial aperture.

Discussion

Morphogenesis of Pleurobranchaea maculata

Development of *Pleurobranchaea maculata* is similar to that of *P. japonica* (Tsubokawa and Okutani, 1991) in terms of egg mass characteristics and overall larval morphology. However, the present study revealed several additional traits that warrant discussion, including the mantle glands, post-velar cells, sensory organs, and the position of the mouth.

The mantle of *P. maculata* becomes richly glandular as it grows back over the larval shell. These simple, tubular glands project through the entire thickness of the mantle, appear fairly early in ontogeny, and persist through metamorphosis. Thompson and Slinn (1959) and Thompson (1988) reported the secretion of sulfuric acid from the mantle of adult Pleurobranchidae from both simple columnar cells and flask-shaped glands. Adults of *Pleuro-*

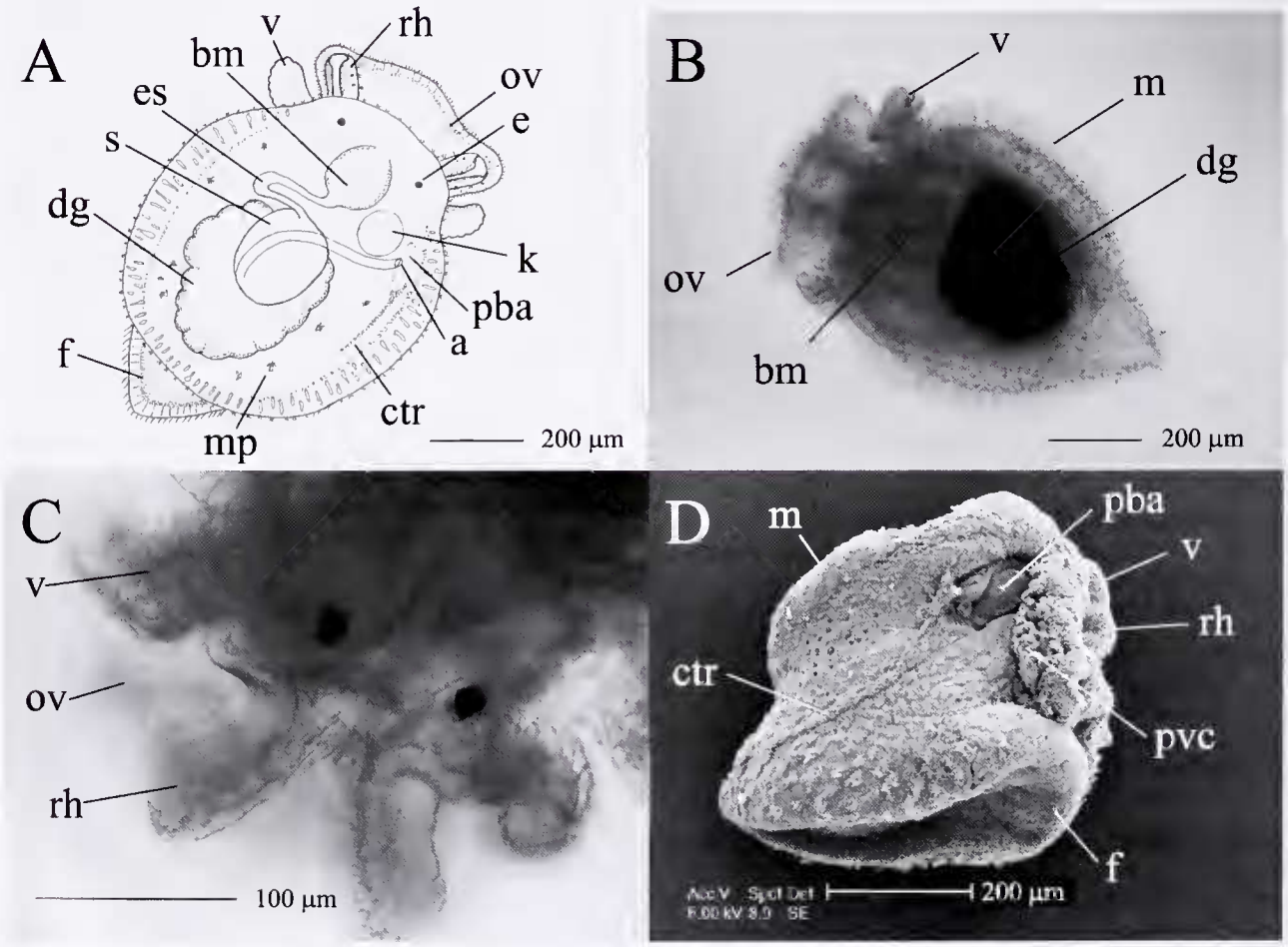

Figure 3. Early juveniles of *Pleurobranchaea maculata*. (A) Newly settled juvenile, dorsal aspect, drawn from life. (B) Bright field micrograph of a newly settled juvenile, ventral aspect. (C) Bright field micrograph of the head. (D) Scanning electron micrograph of a newly settled juvenile. a, anus; bm, buccal mass; ctr, lateral ciliary tract; dg, digestive gland; e, eye; es, esophagus; f, foot; k, kidney; m, mantle; mp, mantle pigment; ov, oral veil; pba, prebranchial aperture; pvc, post-velar cells; rh, rhinophore; s, stomach; v, remnant of velar lobe.

branchaea and *Pleurobranchus* also synthesize a variety of defensive compounds (Ciavatta *et al.*, 1995; Spinella *et al.*, 1997). The abundance and differentiation of mantle glands in *P. maculata* pediveligers suggest that they function in late-stage larvae and likely confer chemical protection to planktonic pediveligers and newly settled juveniles. The capacity of larvae to synthesize their own chemical defense is unusual. Chemical defense occurs in eggs or egg masses of some opisthobranchs; however, in these other taxa, the defensive compounds are maternally derived, as occurs in the Anaspidea (Pennings, 1994), Ascoglossa (Paul and Van Alstyne, 1988), Nudibranchia (Pawlik *et al.*, 1988), and tylodinid Notaspidea (Ebel *et al.*, 1999).

The post-velar cells are also unusual and, to my knowledge, have not been reported elsewhere. These cells develop in larvae, persist through early metamorphosis, and are resorbed with the rest of the velum. The post-velar cells greatly increase the surface area of the lower velar surface through their abundance and highly folded apical surface. While the function of these cells is unknown, it seems reasonable to suggest that they are secretory because of their size, number, and morphology. If secretory, they may protect the head and velum by neutralizing secretions of the mantle glands.

The rhinophores of *P. maculata* originate from the center of the upper velar surface; appear fairly early in larval development; and grow anteriorly as curved, ciliated ridges of tissue. In contrast, the rhinophores of *P. japonica* arise from a pair of depressions at the lateral edges of the oral veil (Tsubokawa and Okutani, 1991). The intravelar location of the rhinophores in *P. maculata* is similar to that of the nudibranch *Rostanga pulchra* MacFarland, 1905 (Chia and Koss, 1982, 1991), although the definitive morphology differs in that the rhinophores of nudibranchs are solid and lack a lateral groove. A cephalic organ, as observed in *P. maculata*, was not reported in *P. japonica* (Tsubokawa and Okutani, 1991) but would be difficult to observe without SEM. However, a cephalic organ is present in some cephalaspids (Schaeffer and Ruthensteiner, 2001) and nudibranchs (Chia and Koss, 1984).

Also of interest is the position of the mouth posterior to the subvelar ridge in pediveligers. Veligers typically have a mouth positioned between the velar cilia (*i.e.*, the pre-oral cilia, or prototroch) and the subvelar ridge (*i.e.*, the post-oral cilia, or metatroch). Whether this is the original embryological location of the mouth (*e.g.*, protostomal) or represents a secondary mouth opening is not known. Comparative work is needed to clarify the embryological origins and potential migration of the definitive mouth from the protostome.

The ciliary tract on the right lateral foot has not been reported elsewhere in notaspideans, but is reported for nudibranchs (*e.g.*, Bonar and Hadfield, 1974; Goddard, 1996). The ciliary tract in *P. maculata*, not present in adults, occupies the position of the adult gill (Willan, 1983). The tract may serve to generate water currents directed away from the prebranchial aperture, which houses the anus and nephroproct; this function is likely taken over by the gill cilia once the gill has formed. Gill formation was not observed in the present study, although Tsubokawa and Okutani (1991) describe the gill in *P. japonica* in early juveniles.

Pattern and process in notaspidean development

Egg mass structure. Egg masses have been described for several species of Notaspidea (Table 2). As is typical of opisthobranchs, eggs are located in capsules within an elongate string that runs in a double spiral around the periphery of a jelly mass, although in some notaspideans the string is poorly defined (Millen, unpubl. obs. cited in Strathmann, 1987) or highly modified (Bandel, 1976). In some species, capsules contain a single egg; in others, as many as 37 eggs per capsule are common (Table 2). Egg size is variable among species and, although the sample size is small, appears correlated with developmental mode (Table 2), as expected for opisthobranchs (Hadfield and Miller, 1987). However, in some Notaspidea, egg size can vary considerably within one species; in the lecithotrophic *Berthellina citrina*, for example, variation in egg size is reported to span 140 μm, ranging from 270 to 410 μm over 15 egg masses (Usuki, 1969).

Larval morphology. Morphogenesis in notaspidean veligers is modified from the pattern—characteristic of benthic opisthobranchs—that is observed in the Cephalaspidea, Ascoglossa, Anaspidea, and Nudibranchia (*e.g.*, Thompson, 1976). Major differences include shell growth and loss, notum formation, and lack of an operculum. Otherwise, morphogenesis appears similar to that of other opisthobranchs.

In most notaspideans (Table 2), larval shells are Type 1 (whorled; typical of most classes of opisthobranchs) or less commonly, Type 2 (inflated and cuplike; occurs in some nudibranchs). However, once the protoconch has formed, growth of the larval shell in pleurobranchids is atypical of other opisthobranchs in mechanism and timing. In other opisthobranchs, the larval shell grows *via* secretions by the mantle fold, a ridge of tissue next to the shell aperture (Tardy, 1991). In pleurobranchids, the mantle fold is lost in early veligers at the same time as the mantle extends past the aperture to ultimately cover the entire larval shell. This occurs after hatching for planktotrophic species of *Pleurobranchaea* (Tsubokawa and Okutani, 1991; this study) or within the egg capsule for lecithotrophic species of *Berthellina* (Usuki, 1969) and direct-developing *Bathyberthella* (Wägele, 1996). Thus larval shell growth is concurrent with mantle overgrowth and occurs despite the migration of the mantle edge away from the aperture; presumably, the region of mantle adjacent to the aperture retains shell-secretion

Table 2

Summary of reproductive data for the Notaspidea

Species	Development Mode*	Egg Size	Egg Mass Shape†	No. Eggs/ Mass	No. Eggs/ Capsule	Veliger Shell Type‡	Time to Hatching (d, °C)	Hatching Size¶	Length of Planktonic Period (d)	Ref.
Umbraculidae										
Umbraculum sinicum (Gmelin, 1791)	P	80 μm	A	10,206,000	30	1	10	—	—	Ostergaard, 1950
	—	88 μm	A	4,500,000	37	—	—	—	—	Thompson, 1970
Tylodinidae										
Tylodina corticalis Tate, 1889	—	98 μm	A	—	—	—	—	—	—	Thompson, 1970
Pleurobranchidae										
Bathyberthella antarctica Willan and Bertsch, 1987	D	—	B	—	1	2	100⁺ d, 0 °C	(1.6 mm)	0	Wägele, 1996
Berthella agassizii (MacFarland, 1909)	—	—	A	70,000	1	—	7 d, —	—	—	Bandel, 1976
Berthella californica (Dall, 1900)	—	—	A	—	—	—	—	—	—	Behrens, 1980
	P	93 μm	A	—	1–2	1	18 d, 12 °C	153 μm	—	Goddard 1984, 2001b
Berthella plumula (Montagu, 1803)	L	200 μm	A	—	—	1	—	—	—	Thompson, 1976
Berthella strongi (MacFarland, 1966)	P	—	B	—	1	1	8 d, 18 °C	131 μm	—	J. Goddard, *pers. comm.*
Berthellina citrina (Rueppell and Leuckart, 1828)	L	~200 μm	A	—	1	1	10 d, 28 °C 21 d, 17 °C	(~280 μm) 400 μm	2–6 d	Gohar and Abul-Ela, 1957
	L	340 μm	A	—	1	1	21 d, 20 °C	(750 μm)	3–6 d	Usuki, 1969
Berthellina engeli Gardiner, 1936	P	—	A	—	6–9	1	—	144 μm	—	J. Goddard, *pers. comm.*
Berthellina quadridens (Moerch, 1863)	—	—	A	50,000	1	—	15 d, —	—	—	Bandel, 1976
Pleurobranchaea californica MacFarland, 1966	P	—	—	—	—	1	—	180	—	Goddard, 2001b
Pleurobranchaea japonica Thiele, 1925	P	100 μm	B	530,000	7	1	6 d, 21 °C	152 μm	15 d	Tsubokawa and Okutani, 1991
Pleurobranchaea maculata (Quoy and Gaimard, 1832)	P	—	B	—	—	—	10 d, —	180 μm	6 d	Willan, 1983
	P	100 μm	B	318,400	4	1	8 d, 20 °C	135 μm	21 d	present study
Pleurobranchus membranaceus Montagu, 1811	P	88 μm	A	1,795,500	1	1	—	—	—	Tchang Si, 1931
Pleurobranchus testudinarius Cantraine, 1835	—	—	A	300,000	1	—	10 d, —	—	—	Bandel, 1976

* Modes of larval development include planktotrophy (P), lecithotrophy (L) or direct development (D).

† Classification of egg masses follows that of Hurst (1967) and includes ribbons (A) and cylindrical cords (B).

‡ Larval shell type follows Thompson (1961) as whorled (1) or inflated (2).

¶ Hatching size refers to shell length unless given in parentheses, when it refers to body length.

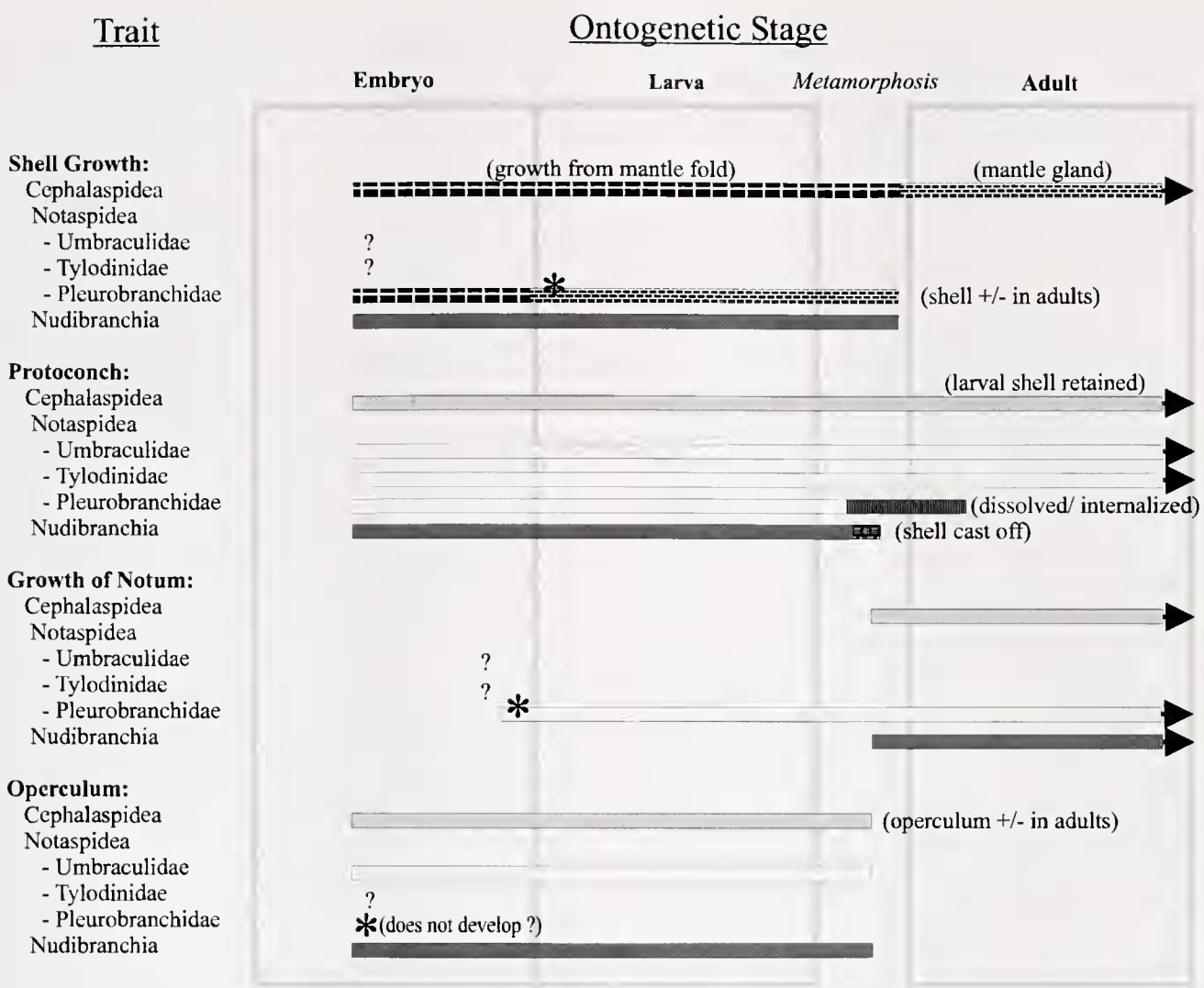

Figure 4. Heterochrony and pleurobranchid development. Data summarize the time of onset and offset of shell growth, protoconch (formation/loss), notum growth, and operculum (formation/loss). Timing is generalized to ontogenetic stage (embryo, larva, and adult) for Cephalaspidea (light gray bars), Notaspidea (white bars), and Nudibranchia (dark gray bars). Different patterns within a bar indicate differences in the developmental process underlying a particular trait; for example, shell growth occurs at the mantle fold in early stages and the mantle gland in later stages, and shell loss occurs through dissolution (some pleurobranchids) or being cast off (Nudibranchia). * Denotes a change in time of onset in pleurobranchids, relative to other indicated groups. +/− Indicates that the trait is present in the adults of only some species in each family or order. ? Indicates that time of onset is not known. References are provided in the text and in Table 2.

activity in larvae. Migration of the mantle edge away from the aperture suggests that shell growth in pleurobranchids is modified in several ways. First, shell growth in larvae occurs by means of the early onset of an ontogenetic process found in juveniles and adults of species that retain their shells (Fig. 4). Second, morphogenesis of the shell and mantle are decoupled from other morphological changes that occur during metamorphosis. Third, shell-less notaspideans have retained the plesiomorphic mechanism of adult shell overgrowth found in shelled notaspideans. This mechanism of shell overgrowth is also distinct from the growth of epipodial (*e.g.*, Anaspidea) or parapodial (*e.g.*, Cephalaspidea) lobes in juveniles of other shelled opisthobranchs (Tardy, 1991).

In shell-less notaspideans, the mechanism of loss of the larval shell is also atypical of opisthobranchs (Fig. 4). In other opisthobranch orders, the retractor muscles are severed and the shell cast off at metamorphosis (*e.g.*, Ascoglossa, Nudibranchia, Gymnosomata). In contrast, the shell-less Pleurobranchidae lose the larval shell through overgrowth and dissolution by the mantle during or shortly after metamorphosis (Tsubokawa and Okutani, 1991). This

distinctive mechanism of shell loss supports the hypothesis that in the Notaspidea—as has been suggested throughout the opisthobranchs (Gosliner, 1994)—shell loss is apomorphic. It additionally suggests that similarities among adults in shell loss and notum morphology have evolved through homoplasy in Notaspidea and Nudibranchia.

Mantle growth in notaspideans is also atypical. In most opisthobranchs the mantle remains small until after metamorphosis. In contrast, the mantle in Notaspidea begins to take on adult characteristics at an early larval stage. This early development includes formation of the notum (Gohar and Abul-Ela, 1957; Thompson, 1976; Tardy, 1991; Tsubokawa and Okutani, 1991; Wägele, 1996) and also the differentiation of cilia and glands (present study). Early onset of notum differentiation may provide the larva and newly settled juvenile with an effective means of defense (*e.g.*, potential acid cells), increased sensory perception (*e.g.*, mantle ciliary tufts), and a larger size, with the associated advantages of predator deterrence and increased buoyancy.

Larvae of pleurobranchid notaspideans lack an operculum. The single recorded exception is Willan's (1983) note of an operculum in *P. maculata;* however, an operculum was not observed in the present study of this species. Lack of an operculum may be characteristic only of pleurobranchids, however, as larvae of the tylodinid *Umbraculum sinicum* (Gmelin, 1791) is described as having an operculum (Ostergaard, 1950). Larvae of all other opisthobranch orders have an operculum, with individual exceptions such as the nudibranch *Aegires albopunctatus* MacFarland, 1905 (Goddard, 2001a). The operculum is lost at metamorphosis in most opisthobranchs, except for a few species of Cephalaspidea (Thompson, 1976). This suggests that loss of an operculum in pleurobranchid larvae is an apomorphic trait that represents an earlier (*i.e.*, embryonic) onset of a condition common to the adults of other orders (Fig. 4).

As with other opisthobranchs, the mantle cavity of notaspidean veligers contains several cell clusters that have been referred to as "larval kidneys" and "anal cells" (Gohar and Abul-Ela, 1957; Usuki, 1969; Tsubokawa and Okutani, 1991). The function and ultrastructure of these organs are unknown, and the terms are used inconsistently across gastropod taxa. Thus, it is more appropriate to use the descriptor "pigmented mantle organ" to refer to the darkly pigmented structure associated with the anus. A pigmented mantle organ is found in the planktotrophic veligers of several notaspideans, including *P. californica* MacFarland, 1966 (Goddard, 2001a), *P. japonica* (Tsubokawa and Okutani, 1991), *P. maculata* (present study), *Berthella californica* (Dall, 1900) (Goddard, 1984), *B. strongi* (MacFarland, 1966) (Goddard, pers. comm.), *Berthellina engeli* Gardiner, 1936 (Goddard, pers. comm.) and *Umbraculum sinicum* (Ostergaard, 1950). A similar organ is found in planktotrophic species of Cephalaspidea. A pigmented mantle organ is lacking in non-planktotrophic notaspideans, including *Berthellina citrina* (Usuki, 1969), the probably lecithotrophic *Berthella plumula* (Montagu, 1803) (Thompson, 1976) and the direct-developing *Bathyberthella antarctica* Willan and Bertsch, 1987 (Wägele, 1996). Although the pigmented mantle organ appears similar in the Notaspidea and Cephalaspidea, ultrastructural and detailed embryological studies are needed to determine if they are homologous. All three studied species of *Pleurobranchaea* (*P. japonica, P. californica,* and *P. maculata;* Tsubokawa and Okutani, 1991; Goddard, 2001b; present study) also have a large, transparent organ positioned adjacent and dorsal to the pigmented mantle organ, which is here considered to be the rudiment of the kidney (following Gohar and Abul-Ela, 1957).

Settlement and metamorphosis. Pediveligers of *Berthellina citrina, Pleurobranchaea japonica,* and *P. maculata* all settle on biofilmed culture dishes (Gohar and Abul-Ela, 1957; Usuki, 1969; Tsubokawa and Okutani, 1991; present study). Preferences for specific substrates were not tested, but a nonspecific cue is probable as all three species are opportunistic carnivores.

Settlement and metamorphosis are also decoupled events in pleurobranchids. Whereas settlement (acquisition of a benthic lifestyle) occurs over a relatively short time span, metamorphosis (the acquisition of an adult morphology) begins early in larval life with an accelerated onset of some processes of differentiation typical of adults (*e.g.*, shell and notum growth), while differentiation of other organ systems (*e.g.*, velum, foot) appears similar to that of other opisthobranch orders. For example, the larval shell is produced by a mantle region other than the mantle fold; thus we see an early onset of a shell-growth mechanism common in juveniles and adults of shelled species (Fig. 4). Also, in the mantle of early larvae, "adult" structures such as glands and external cilia undergo rapid growth and differentiation processes that also are associated with juvenile development in other orders. Absence of an operculum in pleurobranchid larvae is also a trait typical of adult opisthobranchs. Collectively, these observations suggest that in the Pleurobranchidae, aspects of the specialized morphology of the adult have evolved through heterochronic changes in specific morphogenetic events associated with metamorphosis.

Phylogenetic implications

Development in the Notaspidea is relatively poorly known, and the data we have primarily includes the Pleurobranchidae, while details are lacking for the Umbraculidae and Tylodinidae. The descriptions of development that are available for the Pleurobranchidae support the current hypothesis that the Notaspidea are phylogenetically closely linked with the Nudibranchia (Schmekel, 1985; Gosliner, 1994; Wägele and Willan, 2000) yet share some, possibly plesiomorphic, traits with the Cephalaspidea. Potentially

plesiomorphic larval traits include the pigmented mantle organ (common to planktotrophic species of the Cephalaspidea and Notaspidea), the cephalic sensory organ (found in Cephalaspidea and Nudibranchia), and a type 1 larval shell (common to all orders).

Synapomorphies with the Nudibranchia include the shape of the egg mass and the presence of rhinophores that arise from the upper velar field. This agrees with Wägele and Willan (2000), who suggest that similarities in innervation of the rhinophores support homology in the Pleurobranchoidea and Nudibranchia, despite differences in morphology (rolled *versus* solid structure, respectively). Apomorphies in the Pleurobranchidae include lack of the larval operculum, the mechanism of shell loss or internalization, and the pattern of notum formation. These apomorphies have evolved through heterochrony, manifest as an early (*i.e.*, embryonic or larval) onset of developmental processes that typically occur in juveniles of other orders (Fig. 4). Understanding the phylogenetic relevance of novel traits shown by *P. maculata* (*e.g.*, post-velar cells, position of the mouth) awaits further, comparative embryological and ultrastructural work.

Acknowledgments

This research was conducted at the Leigh Marine Laboratory, University of Auckland, New Zealand. I thank the Leigh staff, particularly B. Dobson, for their support. Specimens were examined with SEM at the Research Centre for Surface and Materials Science at the University of Auckland. I especially thank C. Hobson (School of Engineering), A. Turner (School of Biological Sciences), and B. James (School of Engineering) for their kind assistance and providing access to their lab facilities. J. Goddard generously provided unpublished observations and insight into opisthobranchs, which have considerably added to the manuscript. This manuscript benefited from the comments of an anonymous reviewer and of M. Gibson and I. Paterson. J. Havenhand and L. Page provided insight on veliger structure and physiology. This research was supported by the Natural Science and Engineering Research Council of Canada (NSERC).

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